

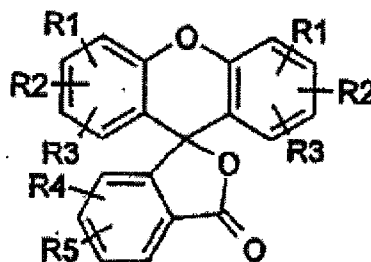
## AMENDMENTS TO THE CLAIMS

This listing of claims replaces all prior versions and listings of claims in the application:

### Listing of claims:

1-22. (Cancelled)

23. (Withdrawn) A phthalein of general formula (I):



(I)

wherein R1, R2, R3, R4 and R5, which are identical to or different from one another, are selected from the group consisting of hydrogen, hydroxyl, halogen, acetyl, amino, phosphate, nitro, sulfonate, carboxyl, alkylcarboxyl having from 2 to 30 carbon atoms, alkyl having from 1 to 30 carbon atoms, cycloalkyl having from 3 to 12 carbon atoms, alkyloxy having from 1 to 30 carbon atoms, haloalkyl having from 1 to 30 carbon atoms, hydroxyalkyl having from 1 to 30 carbon atoms, alkyl ester having from 2 to 40 carbon atoms, nitroalkyl having from 1 to 30 carbon atoms, carboxyalkyl having from 2 to 30 carbon atoms, aminoalkyl having from 1 to 30 carbon atoms, sulfoalkyl having from 1 to 30 carbon atoms, aryl, aryloxy, arylalkyl, haloaryl, aryl ester, succinimidyl ester, isothiocyanate, maleimide, iodoacetamide, haloacetamide, chlorosulfonic, purine or pyrimidine bases, monosaccharides, preferably hexoses or pentoses, oligosides and polysides, polypeptides, proteins and phospholipids,

R3 and R5 are not hydrogen when R1 is a group  $-\text{CH}_2-\text{CH}_2-\text{COOH}$ , R2 is a hydroxyl group and R4 is a group  $-\text{COOH}$ ,

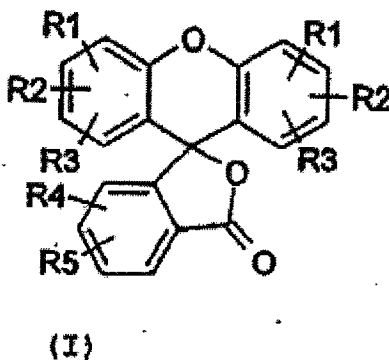
these phthaleins containing no more than 1% by weight of residual impurities.

24. (Withdrawn) The phthalein as claimed in claim 23 containing no more than 0.5% by weight of residual impurities.

25. (Withdrawn) The phthalein as claimed in claim 24 containing no more than 0.2% by weight of residual impurities.

26. (Withdrawn) The phthalein as claimed in claim 23 consisting of fluorescein.

27. (Currently amended) A method for preparing phthaleins, wherein the residual impurities have been removed, having the general formula (I):

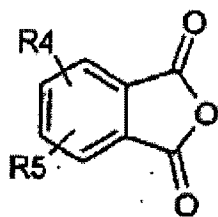


wherein R1, R2, R3, R4 and R5, which are identical to or different from one another, are selected from the group consisting of hydrogen, hydroxyl, halogen, acetyl, amino, phosphate, nitro, sulfonate, carboxyl, alkylcarboxyl having from 2 to 30 carbon atoms, alkyl having from 1 to 30 carbon atoms, cycloalkyl having from 3 to 12 carbon atoms, alkyloxy having from 1 to 30 carbon atoms, haloalkyl having from 1 to 30 carbon atoms, hydroxyalkyl having from 1 to 30 carbon atoms, alkyl ester having from 2 to 40 carbon atoms, nitroalkyl having from 1 to 30 carbon atoms, carboxyalkyl having from 2 to 30 carbon atoms, aminoalkyl having from 1 to 30 carbon atoms, sulfoalkyl having from 1 to 30 carbon atoms, aryl, aryloxy, arylalkyl, haloaryl, aryl ester, succinimidyl

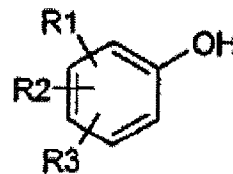
ester, isothiocyanate, maleimide, iodoacetamide, haloacetamide, chlorosulfonic, purine or pyrimidine bases, monosaccharides, preferably hexoses or pentoses, oligosides and polyosides, polypeptides, proteins and phospholipids,

R3 and R5 are not hydrogen when R1 is a group  $-\text{CH}_2-\text{CH}_2-\text{COOH}$ , R2 is a hydroxyl group and R4 is a group  $-\text{COOH}$ ,

wherein a phthalic anhydride derivative of formula (II) is condensed with a phenol or naphthol compound of formula (III)



(II)



(III)

in which R1, R2, R3, R4 and R5 have the same meanings as above, the condensation being carried out in a solvent consisting of an organic acid ester of formula (IV)



wherein R<sub>6</sub> is selected from the group consisting of hydrogen, alkyl having from 1 to 30 carbon atoms, cycloalkyl having from 3 to 12 carbon atoms, haloalkyl having from 1 to 30 carbon atoms, hydroxyalkyl having from 1 to 30 carbon atoms, nitroalkyl having from 1 to 30 carbon atoms, aryl, aryloxy, alkylaryl, arylalkyl, substituted arylalkyl, haloaryl, aryl ester, alkyl ester having from 2 to 40 carbon atoms, and alkyloxy having from 1 to 30 carbon atoms, R<sub>7</sub> being selected from the group consisting of alkyl having from 1 to 30 carbon atoms, cycloalkyl having from 3 to 12 carbon atoms, haloalkyl having from 1 to 30 carbon atoms, hydroxyalkyl having from 1 to 30 carbon atoms, nitroalkyl having from 1 to 30 carbon atoms, aryl, aryloxy, alkylaryl, arylalkyl,

substituted arylalkyl, haloaryl, aryl ester, alkyl ester having from 2 to 40 carbon atoms, or alkyloxy having from 1 to 30 carbon atoms.

28. (Previously presented) The method as claimed in claim 27, wherein the compound of formula (III) is selected from the group consisting of resorcinol, orcinol, naphthol, pyrogallol, alkylaminophenol and arylaminophenol.

29. (Withdrawn) The method as claimed in claim 27, wherein the solvent is an organic acid ester of formula (IV)



wherein  $R_6$  is selected from the group consisting of hydrogen, alkyl having from 1 to 30 carbon atoms, cycloalkyl having from 3 to 12 carbon atoms, haloalkyl having from 1 to 30 carbon atoms, hydroxyalkyl having from 1 to 30 carbon atoms, nitroalkyl having from 1 to 30 carbon atoms, aryl, aryloxy, alkylaryl, arylalkyl, substituted arylalkyl, haloaryl, aryl ester, alkyl ester having from 2 to 40 carbon atoms, and alkyloxy having from 1 to 30 carbon atoms,  $R_7$  being selected from the group consisting of alkyl having from 1 to 30 carbon atoms, cycloalkyl having from 3 to 12 carbon atoms, haloalkyl having from 1 to 30 carbon atoms, hydroxyalkyl having from 1 to 30 carbon atoms, nitroalkyl having from 1 to 30 carbon atoms, aryl, aryloxy, alkylaryl, arylalkyl, substituted arylalkyl, haloaryl, aryl ester, alkyl ester having from 2 to 40 carbon atoms, or alkyloxy having from 1 to 30 carbon atoms.

30. (Currently amended) The method as claimed in claim 27, wherein the organic acid ester is selected from the group consisting of methyl benzoate, ethyl benzoate, propyl benzoate, [[or]] butyl benzoate, methyl heptanoate, ethyl heptanoate, propyl heptanoate, [[or]] butyl heptanoate, methyl octanoate, ethyl octanoate, propyl octanoate [[or]] butyl octanoate, methyl laurate, ethyl laurate, propyl laurate, [[or]] butyl laurate,

methyl myristate, ethyl myristate, propyl myristate, [[or]] butyl myristate or methyl palmitate, ethyl palmitate, propyl palmitate, [[or]] butyl palmitate, and mixtures thereof.

31. (Previously presented) The method as claimed in claim 27, wherein the condensation reaction is carried out at between 150°C and 250°C, optionally under pressure.

32. (Previously presented) The method as claimed in claim 27, wherein the reaction is carried out in the presence of a catalyst selected from the group consisting of Lewis acids, such as  $\text{ZnCl}_2$  or  $\text{AlCl}_3$ , Brönsted acids such as  $\text{H}_2\text{SO}_4$  or polyphosphoric acid.

33. (Previously presented) The method as claimed in claim 32, wherein the catalyst is an alkali metal hydrogen sulfate.

34. (Previously presented) The method as claimed in claim 33, wherein the catalyst is potassium hydrogen sulfate ( $\text{KHSO}_4$ ) or sodium hydrogen sulfate ( $\text{NaHSO}_4$ ).

35. (Previously presented) A method for acidifying the product resulting from the condensation of a phthalic anhydride derivative of formula (II) with a phenol or naphthol compound of formula (III), the formulae (II) and (III) being those of claim 27, wherein the reaction is carried out in an anhydrous organic medium, by the addition of a strong acid or one of its precursors, selected from the group consisting of sulfuric acid, hydrochloric acid, hydrobromic acid, hydrofluoric acid, hydriodic acid, polyphosphoric acid, pyrophosphate ( $\text{P}_2\text{O}_5$ ), and mixtures thereof, the acidification being carried out until the phthalein crystals resulting from the condensation are converted to phthalein crystals having a different structure.

36. (Withdrawn) The method as claimed in claim 35, wherein the condensation product is the product obtained by the method as claimed in claim 27.

37. (Previously presented) The method as claimed in claim 35, comprising a step consisting in washing the product obtained after acidification, said washing step being carried out with a washing solution selected from the group consisting of water, alcohols, ketones, ethers and halogenated solvents, pure or as a mixture, until the crystals are reconverted to the structure that they had before the acidification reaction.

38. (Currently amended) A method for preparing a fluorescein having a purity such that its content of each of the by-products of the reaction is less than or equal to 0.2%, the sum of the contents of each of these by-products being less than or equal to 0.5%, said method comprising the following successive steps:

condensing phthalic anhydride with resorcinol, in a solvent consisting of an ester of an aliphatic or aromatic organic acid, in the presence of a catalyst selected from the group consisting of Lewis acids or Brönsted acids, said ester of an aliphatic or aromatic organic acid having the formula (IV)



wherein R<sub>6</sub> is selected from the group consisting of hydrogen, alkyl having from 1 to 30 carbon atoms, cycloalkyl having from 3 to 12 carbon atoms, haloalkyl having from 1 to 30 carbon atoms, hydroxyalkyl having from 1 to 30 carbon atoms, nitroalkyl having from 1 to 30 carbon atoms, aryl, aryloxy, alkylaryl, arylalkyl, substituted arylalkyl, haloaryl, aryl ester, alkyl ester having from 2 to 40 carbon atoms, and alkyloxy having from 1 to 30 carbon atoms, R<sub>7</sub> being selected from the group consisting of alkyl having from 1 to 30 carbon atoms, cycloalkyl having from 3 to 12 carbon atoms, haloalkyl having from 1 to 30 carbon atoms, hydroxyalkyl having from 1 to 30 carbon atoms, nitroalkyl having from 1 to 30 carbon atoms, aryl, aryloxy, alkylaryl, arylalkyl, substituted arylalkyl, haloaryl, aryl ester, alkyl ester having from 2 to 40 carbon atoms, or alkyloxy having from 1 to 30 carbon atoms,

suspending the red-colored crystals obtained in the preceding step in an anhydrous solvent selected from the group consisting of alcohols such as absolute ethanol, ketones such as acetone, ethers, halogenated solvents, or mixtures thereof,

acidifying the suspension thus obtained by the addition of a strong acid or one of its precursors, selected from the group consisting of sulfuric acid, hydrochloric acid, hydrobromic acid, hydrofluoric acid, hydriodic acid, polyphosphoric acid, pyrophosphate ( $P_2O_5$ ), and mixtures thereof, until the red-colored crystals are converted to yellow-colored crystals ~~exhibiting the X-ray diffraction analysis of FIG. 2,~~

washing the crystals obtained with a washing solution selected from the group consisting of water, alcohols, ketones, ethers and halogenated solvents, pure or as a mixture, this washing being continued until the yellow-colored crystals are reconverted to red-colored crystals.

39. (Previously presented) The method for preparing a fluorescein as claimed in claim 38, having a purity such that its content of each of the by-products of the reaction is less than or equal to 0.1%.

40. (Previously presented) The method for preparing a fluorescein as claimed in claim 38, wherein the solvent used in the condensation reaction is the ethyl or methyl benzoate or ethyl or methyl palmitate.

41. (Previously presented) The method for preparing a fluorescein as claimed in claim 37, wherein the catalyst used for the condensation reaction is an alkali metal hydrogen sulfate.

42. (Currently amended) The method for preparing a fluorescein as claimed in claim 40, wherein the catalyst is potassium hydrogen sulfate or sodium hydrogen sulfate.

43. (Previously presented) The method as claimed in claim 37, wherein the acidification is carried out by sparging gaseous hydrochloric acid into the phthalein suspension or by the action, on this phthalein, of hydrochloric acid in solution in the anhydrous organic solvent, preferably an alcohol, a ketone, an ether or a halogenated solvent, used alone or as a mixture, even more preferably isopropanol, absolute ethanol or acetone, pure or as a mixture.

44. (Previously presented) A yellow-colored fluorescein crystal having the X-ray diffraction analysis of FIG. 2.

45. (Previously presented) A yellow-colored 4',5'-dimethylfluorescein crystal having the X-ray diffraction analysis of FIG. 4.

46. (Previously presented) A reddish-brown- or mahogany-colored 4',5'-dihydroxyfluorescein crystal having the X-ray diffraction analysis of FIG. 6.

47. (Currently amended) A phthalein crystal obtained by means of the method as claimed in claim [[27]]35.

48. (Currently amended) A fluorescein crystal obtained by means of a method as claimed in claim [[27]]35.

49. (Currently amended) A 4',5'-dimethylfluorescein crystal obtained by means of a method as claimed in claim [[27]]35.

50. (Currently amended) A 4',5'-dihydroxyfluorescein crystal obtained by means of a method as claimed in claim [[27]]35.

51. (Cancelled)



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52. (Currently amended) Pharmaceutical composition for diagnosis, especially for medical imaging comprising the fluorescein crystals obtained according to the method of claim [[27]]35.

53. (Cancelled)

54. (Currently amended) Labeling composition for biotechnological applications comprising the fluorescein crystals obtained according to the method of claim [[27]]35.